EFFECT OF TEMPERATURE ON THE COMPOSITION OF FATTY ACIDS IN *ESCHERICHIA COLI*

ALLEN G. MARR AND JOHN L. INGRAHAM

Department of Bacteriology, University of California, Davis, California

Received for publication July 19, 1962

ABSTRACT

MARR, ALLEN G. (University of California, Davis) AND JOHN L. INGRAHAM. Effect of temperature on composition of fatty acids in *Escherichia coli*. J. Bacteriol. **84**:1260–1267. 1962.—Variations in the temperature of growth and in the composition of the medium alter the proportions of individual fatty acids in the lipids of *Escherichia coli*. As the temperature of growth is lowered, the proportion of unsaturated fatty acids (hexadecenoic and octadecenoic acids) increases. The increase in content of unsaturated acids with a decrease in temperature of growth occurs in both minimal and complex media. Cells harvested in the stationary phase contained large amounts of cyclopropane fatty acids (methylenehexadecanoic and methylene octadecanoic acids) in comparison with cells harvested during exponential growth. Cells grown in a chemostat, limited by the concentration of ammonium salts, show a much higher content of saturated fatty acids (principally palmitic acid) than do cells harvested from an exponentially-growing batch culture in the same medium. Cells grown in a chemostat, limited by the concentration of glucose, show a slightly higher content of unsaturated fatty acids than cells from the corresponding batch culture. The results do not indicate a direct relation between fatty acid composition and minimal growth temperature.

It is a common observation that the lipids of poikilothermic organisms vary with the temperature of growth. Plants (Howell and Collins, 1957), insects (Fraenkel and Hopf, 1940), and microorganisms (Terroine, Hatterer, and Roehrig, 1930; Gaughran, 1947) all appear to contain increased proportions of unsaturated fatty acids or more highly unsaturated fatty acids if they are grown at low temperatures. Homiothermic organisms also contain a greater proportion of unsaturated fatty acids in the surface lipids if the environmental temperature is low (Dean and Hilditch, 1933).

Stemming from these observations, the concept has been developed that composition of the lipid may set the limits of temperature for growth of microorganisms. Gaughran (1947), who investigated the lipid composition of steno- and eurithermophilic bacteria, suggested that cells can not grow at temperatures below the solidification point of their lipids; i.e., the temperature at which the lipids solidify is the minimal temperature for growth. Heilbrunn (1924) and Bělehrádek (1931) proposed the antithetical argument that melting of lipids at high temperature destroys essential structures of the cell; i.e., the temperature at which the lipid melts is the maximal temperature for growth.

The variation in the composition of lipids of microorganisms with temperature of growth has been established by measurement of iodine number, as a means of estimating the proportion of unsaturated fatty acids. The development of the technique of gas-liquid chromatography permits the quantitative measurement of individual fatty acids. In this investigation we determined individual fatty acids in *Escherichia coli* by gas-liquid chromtography. The results show a progressive increase in saturated fatty acids and a corresponding decrease in unsaturated fatty acids as the temperature of growth is increased. However, the results suggest that fatty acid composition is not directly related to the limits of temperature of growth.

MATERIALS AND METHODS

Media. The basal medium was medium 56 of Monod, Cohen-Bazire, and Cohn (1951). Glucose or succinate (0.2%) was added as a carbon source for minimal media. Complex media were prepared by supplementing the glucose-minimal medium with 0.2% Casamino Acids (Difco) or with 0.2% yeast extract (Difco). The Casamino Acids and yeast extract were extracted with diethyl ether to remove lipids.

Organism. E. coli ML30, obtained from Jacques Monod, was used in all these experiments.

Growth conditions. Batch cultures were grown in 5-liter bottles containing 3 liters of medium. The cultures were immersed in water held at the stated temperature ± 0.05 C and sparged through a sintered-glass thimble with air saturated with water vapor at the temperature of the culture.

Continuous cultures were grown in a chemostat operated aerobically at 30 C at a specific dilution rate of 0.20 hr^{-1} (Marr and Marcus, 1962). For carbon limitation, the concentration of glucose in the minimal medium was decreased to 0.05%; and for nitrogen limitation, the concentration of $(NH_4)_2SO_4$ was decreased to 0.0185%. The overflow from the culture was collected in a receiver immersed in an ice bath.

Analysis of fatty acids. Cells were harvested by centrifugation and washed twice with water. Approximately 1 g (dry wt) of cells was hydrolyzed for 2 hr at 120 C in a sealed tube containing 15 ml of 2 N HCl. The hydrolysate was extracted three times with 2 vol of diethyl ether. The combined ether extract was dried with anhydrous Na_2SO_4, and the ether was evaporated by a stream of N_2. The residue was esterified in 5 ml of 0.2 N HCl in anhydrous methanol under reflux for 2 hr. After evaporating the solution to 1 ml under a stream of N_2, 2 ml of water were added; and the methyl esters were extracted with three successive 5-ml portions of petroleum ether. The extract was dried with anhydrous Na_2SO_4, and the petroleum ether was evaporated under a stream of N_2. The residue was dissolved in dry benzene.

The methyl esters were determined by gas-liquid chromatography on a column (0.25 in. by 9 ft) of 25% diethylene glycol-succinate polyester on fire brick at 189 C (Kaneshiro and Marr, 1961).

The amount of each component was computed from the area of the recorded peak, which was estimated from the area of the inscribed triangle. Over the range of concentrations encountered in the analysis of samples, the relationship between area and amount of a given methyl ester was found to be linear; however, the area obtained with different esters was not related linearly either to mole fraction or to weight fraction. Under the conditions used in the analysis, the relative areas corresponding to equal weights of methyl esters were: myristate, 1.1; palmitate and hexadecenoate, 1.0; octadecenoate (oleate), 0.825; methylene hexadecanoate, 0.90; β-hydroxymyristate, 0.55; methylene octadecanoate, 0.775. The relative areas for methylene hexadecanoate and methylene octadecanoate were not determined experimentally; the areas were estimated by interpolation from their retention volumes.

RESULTS

Identification of the fatty acid esters. The principal fatty acids of *E. coli* were identified by gas-liquid chromatography of the methyl esters

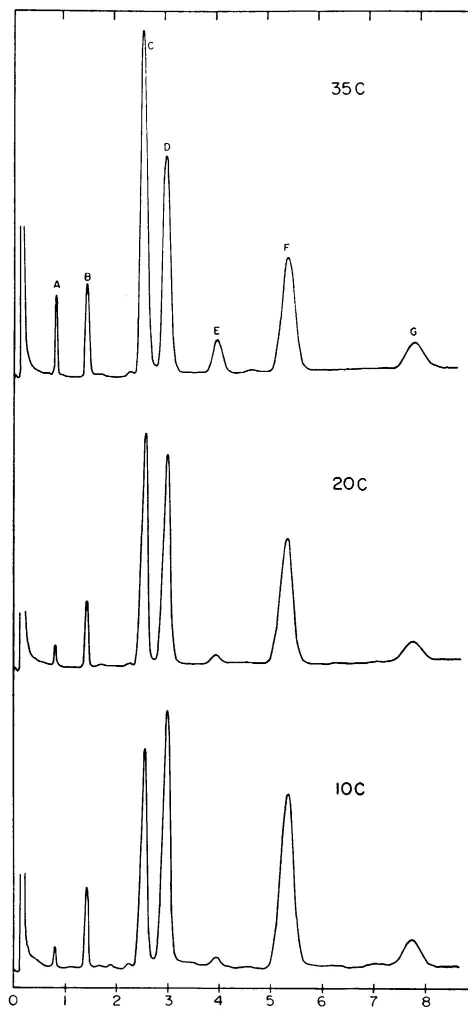

FIG. 1. *Tracings of gas-liquid chromatograms of the methyl esters of fatty acids from Escherichia coli harvested during exponential growth in glucose-minimal medium at 35, 20, and 10 C.* A, *laurate;* B, *myristate;* C, *palmitate;* D, *hexadecenoate;* E, *methylene hexadecanoate;* F, *octadecenoate;* G, *β-hydroxymyristate.*

(Fig. 1) as lauric, myristic, palmitic, hexadecenoic, octadecenoic, methylene hexadecanoic, and β-hydroxymyristic acids. The retention volumes of the esters of the fatty acids from *E. coli* agreed with the retention volume of authentic methyl esters to ± 2%.

The identity of the esters of the unsaturated acids, methyl hexadecenoate and methyl octadecenoate, was confirmed by hydrogenation to methyl palmitate and methyl stearate, respectively. The position of the double bond was not determined; however, the octadecenoic acid of *E. coli* has been reported to be a mixture of *cis*-vaccenic and oleic acids (Kaneshiro and Marr, 1961).

Methyl β-hydroxymyristate was recovered from the mixed methyl esters of *E. coli* by trapping component G from the effluent of the gas-liquid chromatogram (Fig. 1). This methyl ester was purified by chromatography on a column of silicic acid; the ester eluted in the fraction characteristic of methyl esters of hydroxy acids. Methyl β-hydroxymyristate was identified by dehydration and catalytic hydrogenation the product of which was identical with methyl myristate in gas-liquid chromatography. The infrared spectrum of the recovered methyl β-hydroxymyristate was identical with the spectrum of synthetic methyl β-hydroxymyristate, and the retention volumes in gas-liquid chromatography of the recovered ester and synthetic methyl β-hydroxymyristate were identical. Although Ikawa et al. (1953) found β-hydroxymyristic acid as a major component of a polar lipid of *E. coli*, this acid was not detected in the alcohol-soluble lipid of *E. coli* (Kaneshiro and Marr, 1961). Presumably, β-hydroxymyristic acid is released from the bound lipid by acid hydrolysis of the cells.

In many of the samples of the methyl esters from *E. coli*, methyl laurate was only partially resolved from an unidentified component by gas-liquid chromatography, making the quantitative determination of the small amount of methyl laurate difficult (Fig. 1). Because of this difficulty in precise estimation of the amount of methyl laurate, and because lauric acid is a minor component of the lipids of *E. coli*, lauric acid was omitted from all calculations of the composition of the fatty acids. Several minor components are also evident in the chromatograms between the peaks of methyl myristate and methyl palmitate (Fig. 1, 3, 4). These components were not identified, and their areas were not considered in computing the percentages of fatty acids.

Effect of growth temperature on fatty acid composition of cells grown in glucose-minimal medium. Cultures of *E. coli* ML30 were grown in glucose-minimal medium at 10, 15, 20, 25, 30, 35, 40, and 43 C, and were harvested by centrifugation of an exponentially growing culture. Three tracings of representative analyses are shown in Fig. 1. From such analyses, the amount of each fatty acid was computed from the area under each peak (Table 1, Fig. 2). As growth temperature is decreased, the proportion of unsaturated acid increases. The percentage of the most abundant unsaturated fatty acid, octadecenoic acid, decreases continuously with increasing growth temperature over the entire

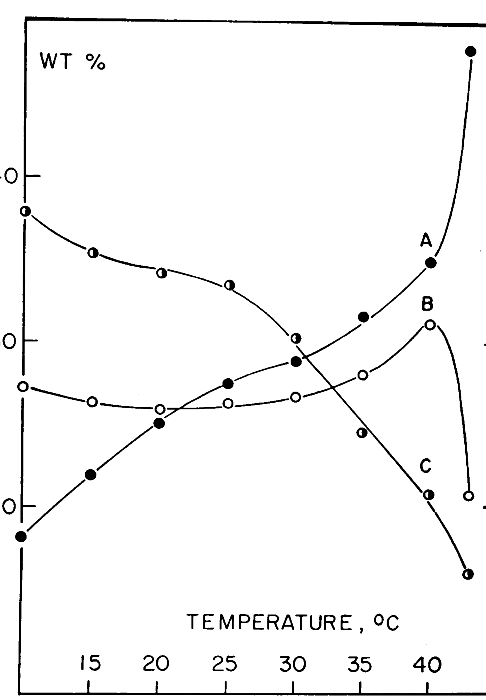

FIG. 2. *Effect of growth temperature on the fatty acid composition of Escherichia coli ML30 harvested during exponential growth in glucose-minimal medium. Per cent by weight of the total fatty acids is calculated as methyl esters, neglecting lauric acid.* A, *palmitic acid;* B, *sum of hexadecenoic acid and methylene hexadecanoic acid;* C, *octadecenoic acid.*

TABLE 1. *Effect of growth temperature on fatty acid composition of Escherichia coli ML30 grown in glucose-minimal medium and harvested during exponential growth*

Fatty acid	Temp (C)							
	10	15	20	25	30	35	40	43
	%	%	%	%	%	%	%	%*
Myristic	3.9	3.8	4.1	3.8	4.1	4.7	6.1	7.7
Palmitic	18.2	21.9	25.4	27.6	28.9	31.7	37.1	48.0
Hexadecenoic	26.0	25.3	24.4	23.2	23.3	23.3	28.0	9.2
Methylene hexadecanoic	1.3	1.1	1.5	3.1	3.4	4.8	3.2	11.6
Octadecenoic	37.9	35.4	34.2	35.5	30.3	24.6	20.8	12.2
Methylene octadecanoic	0	0	0	0	0	0	0	3.7
β-Hydroxymyristic	12.6	12.5	10.4	6.9	10.1	11.0	4.8	7.5
Hexadecenoic-palmitic†	1.43	1.55	0.96	0.84	0.81	0.74	0.75	0.19
Octadecenoic-palmitic†	2.08	1.62	1.35	1.29	1.05	0.78	0.56	0.25

* Calculated as the weight per cent of the methyl ester. Lauric acid is not included in total (see text).
† Ratio of weight per cent.

growth range. The most abundant saturated fatty acid, palmitic acid, increases continuously with increasing growth temperature. The highest rate of change of these fatty acids occurs at high temperature. The percentage of the cyclopropane fatty acid, methylene hexadecanoic acid, also decreases with decreasing growth temperature, and β-hydroxymyristic acid is almost constant. The percentage of hexadecenoic acid increases only slightly at low temperature, but decreases dramatically above 40 C. Ratios of unsaturated to saturated fatty acids (Table 1) serve as useful indices of the degree of shift in fatty acid composition of the cells; e.g., the hexadecenoic-palmitic and octadecenoic-palmitic acid ratios both decrease approximately eightfold over the temperature range of 10 to 43 C.

Effect of growth rate on fatty acid composition. The changes in proportions of fatty acids might have resulted from differences in growth rate imposed by temperature. This possibility was tested by varying the growth rate at constant temperature by altering the composition of the medium. Cultures were grown at 25 C in the following media: succinate-minimal, glucose-minimal, glucose-minimal supplemented with Casamino Acids, and glucose-minimal supplemented with yeast extract.

The cells were harvested during exponential growth and were analyzed to determine the composition of fatty acids (Table 2). The proportion of various fatty acids is somewhat dependent on the medium, but does not correlate with the growth rate (Table 2). The specific growth rate in glucose-minimal medium supplemented with yeast extract at 25 C is almost identical to the growth rate in glucose-minimal medium at 35 C, but the composition of fatty acids of cells grown at 25 C with a supplement of yeast extract (Table 2) corresponds more closely to cells grown in minimal medium (Table 1) at about 10 C as judged by the hexadecenoic-palmitic acid ratio or at about 20 C as judged by the octadecenoic-palmitic acid ratio.

The percentage of palmitic acid is significantly higher in cells grown in glucose-minimal than in the other three media.

Effect of growth temperature on fatty acid composition of cells grown in complex medium. Since cells grown at 25 C in glucose-minimal medium supplemented with yeast extract have a fatty acid composition similar to that of cells grown near the minimal growth temperature in glucose-minimal medium, it was of interest to determine whether growth at low temperature in complex medium would result in an even greater proportion of unsaturated fatty acids. Cultures were grown in glucose-minimal medium supplemented with yeast extract at 10, 20, 25, and 35 C. The cells were harvested during exponential growth and were analyzed for fatty acids (Table 3). The results are comparable to minimal medium except that at any given temperature the percentage of unsaturated fatty acids is higher in complex than in minimal medium. As growth temperature is lowered, the percentage

TABLE 2. *Effect of growth rate on the fatty acid composition of Escherichia coli harvested during exponential growth at 25 C*

Medium	Succinate minimal	Glucose minimal	Glucose minimal + Casamino Acids	Glucose minimal + yeast extract
k (hr^{-1})*	0.35	0.48	0.64	0.88
Equivalent temp†	22 C	25 C	32 C	36 C
	%	%	%	%
Fatty acid				
Myristic	4.2‡	4.4	5.7	4.4
Palmitic	21.0	25.6	24.8	22.2
Hexadecenoic	28.7	27.3	29.6	31.7
Methylene hexadecanoic	1.3	2.1	0.7	0.8
Octadecenoic	34.6	31.5	30.6	31.3
β-Hydroxymyristic	10.2	9.2	8.5	9.6
Ratios of weight per cent				
Hexadecenoic-palmitic	1.34	1.07	1.19	1.43
Octadecenoic-palmitic	1.65	1.23	1.23	1.41

* Specific growth rate, calculated from the equation, $k = \frac{2.303(\log x_2 - \log x_1)}{t_2 - t_1}$ in which x_1 and x_2 are optical densities at times t_1 and t_2.

† Temperature at which the same growth rate is attained in glucose minimal medium.

‡ Calculated as the weight per cent of the methyl ester. Lauric acid is not included in the total (see text).

TABLE 3. *Effect of growth temperature on the fatty acid composition* of Escherichia coli ML30 grown in glucose-minimal medium supplemented with yeast extract*

Fatty acid	Temp of growth (C)			
	10	20	25	35
	%	%	%	%
Myristic	3.9	4.8	5.0	5.6
Palmitic	16.4	22.4	21.8	33.1
Hexadecenoic	30.4	32.9	30.0	28.4
Methylene hexadecanoic	0.7	1.3	1.1	1.8
Octadecenoic	38.8	30.1	31.3	20.2
β-Hydroxymyristic	10.0	8.6	10.9	10.9
Hexadecenoic-palmitic†	1.85	1.47	1.38	0.86
Octadecenoic-palmitic†	2.37	1.34	1.44	0.61

* Calculated as the weight per cent of methyl ester. Lauric acid is not included in the total (see text).

† Ratio of weight per cent.

of palmitic and methylene hexadecanoic acids decrease and the percentage of octadecenoic acid increases. The change in hexadecenoic acid is slight, and the percentage of β-hydroxymyristic acid remains essentially constant.

Fatty acids of cells harvested from cultures in the stationary phase. The preceding measurements differ from earlier reports of the fatty acid composition of *E. coli.* We found only small amounts of methylene hexadecanoic acid and no methylene octadecanoic (lactobacillic) acid except in cells grown near the maximal temperature. Previously, Dauchy and Assilineau (1960) and Kaneshiro and Marr (1961) reported the cyclopropane acids as major components of the lipid of *E. coli.* This apparent discrepancy results from an increase in the cyclopropane acids at the expense of the corresponding unsaturated acids after the end of exponential growth.

Figure 3 shows the analyses of cells harvested from succinate-minimal medium during exponential growth and of cells harvested several hours after the growth of the culture ceased from exhaustion of the carbon source. The percentage of methylene hexadecanoic acid increases approximately 12-fold in the cells from the stationary phase, with a corresponding decrease in hexadecenoic acid. Methylene octadecanoic acid is present in cells from the stationary phase but is not detectable in cells from the culture growing exponentially. A third significant difference is the presence in the cells from the stationary-phase culture of an unresolved component between palmitate and hexadecenoate. This component is clearly revealed in analyses of hydrogenated samples. The retention volume of this component is identical with that of the methyl ester of a branched chain C_{17} acid.

Effect of carbon or nitrogen limitation. The previous experiments suggested a significant influence of nutrition on the proportions of fatty acids produced by *E. coli.* The influence of nutrition and of reduced growth rate was tested

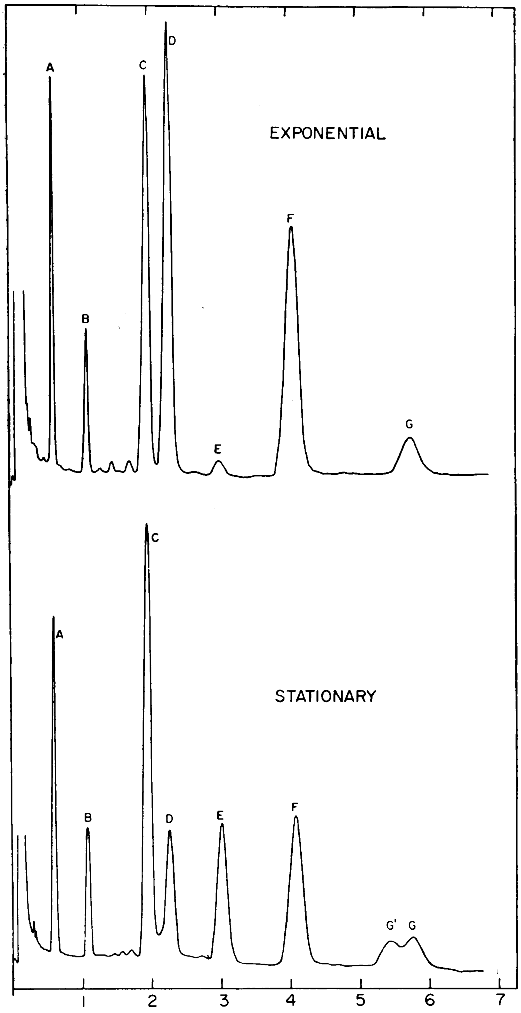

FIG. 3. *Tracing of gas-liquid chromatograms of the methyl esters of fatty acids from Escherichia coli grown in succinate-minimal medium at 25 C and harvested during the exponential phase and during the stationary phase.* A, *laurate;* B, *myristate;* C, *palmitate;* D, *hexadecenoate;* E, *methylene hexadecanoate;* F, *octadecenoate;* G′, *methylene octadecanoate;* G, *β-hydroxymyristate.*

further by cultivation of *E. coli* at 30 C in a chemostat, limited by the concentration either of the carbon source or of the nitrogen source. The flow rate was adjusted to provide a specific growth rate of 0.2 hr^{-1}. The analyses of the cells for fatty acids are shown in Fig. 4. Cells harvested from the glucose-limited culture differ only slightly in fatty acid composition from cells grown in batch culture. Thus, a reduction in specific growth rate from 0.65 to 0.2 hr^{-1} does not significantly alter the proportions of fatty acids. The fatty acid composition of the cells from the nitrogen-limited chemostat is strikingly different. The amount of palmitic and methylene hexadecanoic acids is increased, and the amount of unsaturated fatty acids is decreased. The effect of nitrogen limitation mimics the effect of growth at high temperature.

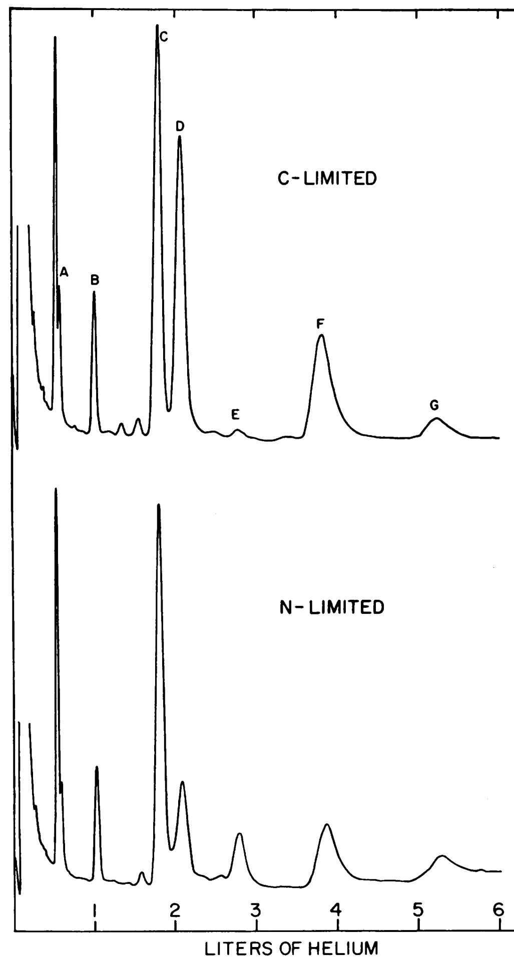

FIG. 4. *Tracings of gas-liquid chromatograms of the methyl esters of fatty acids from Escherichia coli grown in a chemostat at 30 C under conditions of carbon and of nitrogen limitation.* A, *laurate;* B, *myristate;* C, *palmitate;* D, *hexadecenoate;* E, *methylene hexadecanoate;* F, *octadecenoate;* G, *β-hydroxymyristate.*

Changes in fatty acid composition of cells after a shift in temperature from 40 to 10 C. Ng, Ingraham, and Marr (1962) reported that, when a culture of *E. coli* growing exponentially at 40 C is cooled to 10 C, growth ceases for approximately 4 hr, after which the culture grows exponentially for 10 hr at approximately twice the normal growth rate at 10 C. To determine whether the fatty acid composition changes during the lag, cells were harvested just after the period of rapid exponential growth began and were analyzed for fatty acids. The fatty acid composition of these cells differed from the typical composition of cells grown at either 40 or 10 C. The composition was similar to that of cells grown exponentially in the same medium at 25 C, indicating that the fatty acid composition had changed significantly during the period of lag.

DISCUSSION

The proportion of unsaturated fatty acids of *E. coli* decreases continuously as growth temperature is increased in both minimal and complex media. The fatty acids in bacteria are mainly contained in phospholipids, which are an essential part of the structure of the cell membrane rather than mere storage products. Thus, it is tempting to speculate that changes in fatty acid composition with growth temperature are adaptations to environments of different temperature. However, growth at a particular temperature does not result in a unique fatty acid composition, since altering the nutrition independently of temperature also results in major changes in fatty acid composition.

This fact argues against the hypothesis that solidification of lipids sets the minimal growth temperature (Gaughran, 1947). The minimal temperature for growth, which is slightly less than 10 C for *E. coli* ML30 (Ng et al., 1962), is known to be independent of medium. However, the fatty acid composition of cells grown in glucose-minimal medium at 10 C differs markedly from that of cells grown at the same temperature in this medium supplemented with yeast extract. If the fatty acid composition of cells grown in minimal medium did indeed set the minimal temperature for growth, the minimal temperature in complex medium would have been between 15 and 20 C.

The striking difference in fatty acid composition between cells grown at 20 and at 35 C (Table 1) is not demonstrably adaptive. Ng et al. (1962) have shown that the specific growth rate of cultures of *E. coli* ML30 responds immediately to a change in temperature in the range of 20 to 37 C. Cells which have a fatty acid composition characteristic of the steady state of growth at 20 C grow at 37 C at the same rate as cells with a fatty acid composition characteristic of the steady state of growth at 37 C. Thus, rather large changes in the fatty acid composition have no measurable effect on the specific growth rate. The physiological effects of these differences in fatty acid composition appear to be trivial.

The mechanism by which a change in temperature regulates the synthesis of fatty acids has not been established. The effect might be (i) a direct effect of temperature on the relative rates of two or more enzymes which synthesize saturated and unsaturated acids, respectively, (ii) an indirect effect resulting from a change in growth rate, or (iii) an indirect effect resulting from a change in the concentration of intermediates.

The possibility that temperature alters the fatty acid composition through changing the growth rate is not, a priori, an attractive hypothesis, and it can be readily eliminated by the results of the experiments. Increasing the growth rate by supplementation of minimal medium with Casamino Acids or yeast extract results in a higher proportion of unsaturated fatty acids, but increasing the growth rate by increasing temperature results in a lower proportion of unsaturated fatty acids. The most direct demonstration that growth rate, per se, does not control the fatty acid composition comes from a comparison of cells grown in glucose-minimal medium at 30 C in batch culture ($k = 0.64\ hr^{-1}$) and in glucose-limited chemostat ($k = 0.20\ hr^{-1}$). Despite the large difference in growth rates, the fatty acid composition was quite similar.

The effect of nitrogen limitation may offer a clue to the means by which temperature controls the proportions of fatty acids. Limitation of the nitrogen source imposed by cultivation in a chemostat at 30 C with ammonium as the limiting nutrient (Fig. 4) resulted in a dramatic increase in the proportion of palmitic acid at the expense of both hexadecenoic and octadecenoic acids. The fatty acid composition under this condition of nitrogen limitation is equivalent to the composition in a batch culture grown at about 40 C. The increase in proportions of unsaturated fatty acids which results from supplementation of minimal medium with amino acids or yeast extract can also be explained as the relief of a partial deficiency in the intermediates in nitrogen metabolism.

An interesting but unexpected result of this study is the finding that the cyclopropane fatty acids, methylene hexadecanoic and methylene octadecanoic acids, are formed in substantial amounts only after the cessation of exponential growth. Both appear to be formed at the expense of the unsaturated homologue in accord with the hypothesis that the cyclopropane ring is formed by addition of a methylene carbon to an olefin.

ACKNOWLEDGMENTS

This work was aided by a grant from the Whirlpool Corporation, by grant-in-aid RG-6807 from the National Institutes of Health, U.S. Public Health Service, and by grant-in-aid 9860 from the National Science Foundation.

We are indebted to Raymond Combs for technical assistance.

LITERATURE CITED

BĚLEHRÁDEK, J. 1931. Le mechanisme physico-chemique de l'adaptation thermique. Protoplasma **12**:406–434.

DAUCHY, S., AND J. ASSELINEAU. 1960. Sur les acides gras des lipid de *Escherichia coli*. Compt. Rend. **250**:2635–2637.

DEAN, H. K., AND T. P. HILDITCH. 1933. The body fats of the pig. III. The influence of body temperature on the composition of depot fats. Biochem. J. **27**:1950–1956.

FRAENKEL, G., AND H. S. HOPF. 1940. Temperature adaptation and degree of saturation of the phosphatides. Biochem. J. **34**:1085–1092.

GAUGHRAN, E. R. L. 1947. The saturation of bacterial lipids as a function of temperature. J. Bacteriol. **53**:506.

HEILBRUNN, L. V. 1924. The heat coagulation of protoplasm. Am. J. Physiol. **69**:190–199.

HOWELL, R., AND F. I. COLLINS. 1957. Factors affecting linolenic and linoleic acid content of soybean oil. Agron. J. **49**:593–597.

IKAWA, M., J. B. KOEPFLI, S. G. MUDD, AND C. NIEMANN. 1953. An agent from *E. coli* causing hemorrhage and regression of an experimental mouse tumor. III. The component fatty acids of the phospholipide moiety. J. Am. Chem. Soc. **75**:1035–1038.

KANESHIRO, T., AND A. G. MARR. 1961. *Cis*-9,10-methylene hexadecanoic acid from the phospholipids of *Escherichia coli*. J. Biol. Chem. **236**:2615–2619.

MARR, A. G., AND L. MARCUS. 1962. Kinetics of induction of mannitol dehydrogenase in *Azotobacter agilis*. Biochim. Biophys. Acta **64**: 65–82.

MONOD, J., G. COHEN-BAZIRE, AND M. COHN. 1951. Sur la biosynthèse de la β-galactosidase (lactase) chez *Escherichia coli*. La spécificité de l'induction. Biochim. Biophys. Acta **7**:585–599.

NG, H., J. L. INGRAHAM, AND A. G. MARR. 1962. Damage and derepression in *Escherichia coli* resulting from growth at low temperatures. J. Bacteriol. **84**:331–339.

TERROINE, E. F., C. HATTERER, AND P. ROEHRIG. 1930. Les acides gras des phosphatides chez animaux poikilothermes, les végétaux supérieurs et les microorganismes. Bull. Soc. Chim. Biol. **12**:682–702.